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(54) STRETCHED FILM AND CELL CULTURE BASE MATERIAL USING THE SAME

(57)Abstract:

PROBLEM TO BE SOLVED: To provide a stretched film which can be used for cultivating a cell or forming a three-dimensional composition from a cell, and to provide a cell culture base material using the film.

SOLUTION: Hydrophobic organic solvent fluid of a single polymer having biodegradable and amphiphatic properties or a plurality of polymer mixtures is cast on a substrate. The organic solvent is transpired while being condensed on the cast liquid surface, and minute water drops produced by condensation are evaporated to provide a honey-comb structure body. The stretched film is obtained by stretching the honey-comb structure body thus provided.

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## LEGAL STATUS

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CLAIMS

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[Claim(s)]

[Claim 1] The oriented film obtained by extending the honeycomb structure object acquired by evaporating the minute waterdrop which carried out the cast of the hydrophobic organic solvent solution of the polymer mixture which consists of the independent polymer or biodegradability polymer which has biodegradability and amphiphilic, and an amphiphilic polymer on the substrate, was made to dew on this cast liquid front face while transpiring this organic solvent, and was produced by this dew condensation.

[Claim 2] The oriented film according to claim 1 which uses aliphatic series polyester as a biodegradability polymer.

[Claim 3] The oriented film according to claim 1 or 2 which uses the polymer mixture which consists of 50 - 99 w/w% of biodegradability polymer, and 50 - 1 w/w% of amphiphilic polymer as polymer mixture which consists of a biodegradability polymer and an amphiphilic polymer.

[Claim 4] An oriented film given in any of claims 1-3 which carry out the cast of the hydrophobic organic solvent solution on a substrate, are made to dew on this cast liquid front face at the same time it transpires this organic solvent by spraying high humidity air, and are obtained by extending the honeycomb structure object acquired by evaporating the minute waterdrop produced by this dew condensation they are.

[Claim 5] An oriented film given in any of claims 1-4 which carry out the cast of the hydrophobic organic solvent solution on a substrate under atmospheric air of 50 - 95% of relative humidity, are made to dew on this cast liquid front face at the same time it transpires this organic solvent, and are obtained by extending the honeycomb structure object acquired by evaporating the minute waterdrop produced by this dew condensation they are.

[Claim 6] An oriented film given in any of claims 1-5 which extend by uniaxial stretching, biaxial stretching, or triaxial drawing they are.

[Claim 7] An oriented film given in any of claims 1-6 whose rate of expanding of the drawing direction is within the limits of 1.1 to 10 times they are.

[Claim 8] An oriented film given in any of claims 1-7 whose diameter of said honeycomb structure object is 0.1-100 micrometers they are.

[Claim 9] The base material for cell cultures which consists of an oriented film given in any of claims 1-8 they are.

[Claim 10] How to cultivate a cell using an oriented film or the base material for cell cultures according to claim 9 given in any of claims 1-8 they are.

[Claim 11] (1) biodegradability -- and -- amphiphilic -- having -- being independent -- a polymer -- or -- plurality -- a polymer -- mixture -- hydrophobicity -- an organic solvent -- a solution -- a substrate -- a top -- the cast -- carrying out -- this -- an organic solvent -- transpiring -- making -- at the same time -- this -- the cast -- liquid -- a front face -- dewing -- making -- this -- dew condensation -- having been generated -- minute -- waterdrop -- evaporating -- making -- things -- honeycomb structure -- the body -- extending -- a process --; -- and -- ( -- two -- ) -- the above -- honeycomb structure -- the body -- extending -- a process -- : -- containing -- being according to claim 1 -- an oriented film -- manufacture -- an approach .

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DETAILED DESCRIPTION

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[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention relates to the cell culture base material which used an oriented film and it. More specifically, this invention relates to the cell culture base material using an oriented film and it available for formation of a three-dimension organism from culture of a cell, and a cell at a detail about the cell culture base material using an oriented film and it applicable [ with medical care or biotechnology ] to a cell technology and systems engineering with research prosperous in recent years more.

[0002]

[Description of the Prior Art] In the interaction of a cell and an ingredient, it is known that a cell will be influenced not only with the chemical property of a material-list side but with a detailed configuration. Then, when aiming at functional control of a cell from viewpoints, such as systems engineering, processing of the chemical property of the material-list side in contact with a cell and the both sides of detailed structure becomes important. Installation of the size control of the cell adhesion side which used the micro pattern technique used for semiconductor industry etc. as a surface treatment technique as a surface micro-processing method, and the minute slot structure to a substratum, production of the detailed irregularity by the microsphere, etc. are mentioned, and it is known that the surface fine structure will affect growth of a cell etc. greatly.

[0003] A very advanced technique is required for surface setting out using these micro pattern techniques, and the actual condition is having many problems, such as mass production method being impossible and becoming high cost. It is known that the film which has the honeycomb structure of mum scale by carrying out the cast of the dilute solution of the polymer which has structure completely special as another surface patterning technique under high humidity will be obtained. It is the description that this approach is excellent in the profitability which hits to carry out patterning.

[0004] the rod-coil which will specifically become 283 volumes and a page 373 from a hydrophilic block and a hydrophobic block Science and 1999 -- a jib -- the example using the polyphenyl quinoline-block-polystyrene which is a lock polymer -- moreover, the jib which will become 369 volumes and a page 387 from polystyrene and the poly para-phenylene which is an upright block Nature and 1994 -- the example using a lock polymer is indicated. Thus, at the Prior art, the special polymer having a part with the strong autoagglutination force and the part which discovers flexibility was used, these polymers were dissolved in the hydrophobic organic solvent, and the honeycomb structure object was prepared by carrying out the cast of this. On the other hand, this invention persons are SHINSO lids. Films, 1998, 327 to 329 volumes, A page 854, Supra molecular Science, 1998, the 5th volume, A page 331 and molecular crystal liquid crystal, The acrylamide polymer of a hydrophilic property will be made into a principal chain frame in 1998 at the 322nd volume and a page 305. The amphiphilic polymer which has a lactose radical or a carboxyl group as the dodecyl and a hydrophilic side chain as a hydrophobic side chain, Or it has reported giving the thin film which has honeycomb structure by the approach with the same ion complex of anionic polysaccharides, such as heparin and dextran sulfate, and the long-chain

alkyl ammonium salt of the 4th class.

[0005] However, since it was inferior to the self-independence nature of the honeycomb structure object acquired in these polymers or had the fault of honeycomb structure collapsing with time, it was not what offers function sufficient as a base material for cell cultures.

[0006] When performing a cell culture in a cell technology, systems engineering, etc., the base material used as the footing of a cell is required, and it is known in the interaction with a cell like the above-mentioned that a cell will be influenced not only with the chemical property on the front face of best but with a detailed configuration. When aiming at functional control of a cell, a design of the chemical property of a material-list side and the both sides of structure with a detailed cell in contact with a cell becomes important. With the porous film which has honeycomb structure, a honeycomb pattern offers a cell adhesion side and it is shown that porous structure serves as access to the support base of a cell and the supply root of a nutrition.

[0007] If a cell is systematized based on this honeycomb structure film, an artificial organ can be considered as that one usage. However, since embedding inside of the body becomes indispensable when it is made an artificial organ etc., as for this base material, being absorbed to the living body is desirable in the long run. The time amount which a cell culture takes with the ingredient which gives old honeycomb structure maintains structure to stability, and there is nothing that was made from a biodegradability ingredient which is disassembled more than at it. In other words, in combining a honeycomb structure object, a cell technology, and a cell culture technique, and developing to medical-application ways, such as an artificial organ, it is indispensable to use a biodegradability ingredient.

[0008] In view of such a situation, as for this invention persons, a biodegradability polymer the hydrophobic organic solvent solution of a polymer with which 50 – 99 w/w% and an amphiphilic polymer consist of 50 – 1 w/w% It is made to dew on this cast liquid front face at the same time it carries out the cast on a substrate under atmospheric air of 50 – 95% of relative humidity and transpires this organic solvent gradually. The honeycomb structure object acquired by evaporating the minute waterdrop produced by this dew condensation and the film which becomes a list from this honeycomb structure object are proposed (Japanese-Patent-Application-No. No. 340568 [ 11 to ] description (it sets at this event and is un-opening to the public)). However, the array of the pore of the film which has the honeycomb structure produced by this approach was isotropic, and it was difficult to control the array of a cell on this.

[0009]

[Problem(s) to be Solved by the Invention] This invention made it to offer the cell culture base material using an oriented film and it available for formation of a three-dimension organism the technical problem which should be solved from culture of a cell, and a cell. Furthermore, this invention made it the technical problem which should be solved to offer the cell culture base material using the oriented film and it which can control the array of a cell.

[0010]

[Means for Solving the Problem] In order to solve the above-mentioned technical problem, as a result of inquiring wholeheartedly, when this invention persons extended the honeycomb structure object of the elastic polymer which has biodegradability and amphiphilic simulataneously, they found out changing to the array of the pore the honeycomb porosity structure which was an isotropic array indicates an anisotropy to be by this drawing. Furthermore, as a result of cultivating a cardiac muscle cell on this oriented film, it was proved that orientation of the cell could be carried out in accordance with the linear array of pore. This invention is completed based on these knowledge.

[0011] That is, carry out the cast of the hydrophobic organic solvent solution of the polymer mixture which consists of the independent polymer or biodegradability polymer which has biodegradability and amphiphilic, and an amphiphilic polymer according to this invention on a substrate, it is made to dew on this cast liquid front face at the same time it transpires this organic solvent, and the oriented film obtained by extending the honeycomb structure object acquired by evaporating the minute waterdrop produced by this dew condensation is offered.

[0012] In this invention, aliphatic series polyester is preferably used as a biodegradability polymer. In this invention, the polymer mixture which consists of 50 – 99 w/w% of

biodegradability polymer and 50 – 1 w/w% of amphiphilic polymer is preferably used as polymer mixture which consists of a biodegradability polymer and an amphiphilic polymer.

[0013] The oriented film of this invention carries out the cast for example, of the hydrophobic organic solvent solution on a substrate. It is made to dew on this cast liquid front face at the same time it transpires this organic solvent by spraying high humidity air. [ whether it is obtained by extending the honeycomb structure object acquired by evaporating the minute waterdrop produced by this dew condensation, and ] Or the cast of the hydrophobic organic solvent solution is carried out on a substrate under atmospheric air of 50 – 95% of relative humidity. It is made to dew on this cast liquid front face at the same time it transpires this organic solvent, and it is obtained by extending the honeycomb structure object acquired by evaporating the minute waterdrop produced by this dew condensation.

[0014] In this invention, uniaxial stretching, biaxial stretching, or a triaxial drawing can perform a drawing, and the rate of expanding of the drawing direction is within the limits of 1.1 to 10 times preferably, and the diameter of a honeycomb structure object is 0.1–100 micrometers.

[0015] According to another side face of this invention, the base material for cell cultures which consists of an oriented film of above-mentioned this invention is offered. According to still more nearly another side face of this invention, the method of cultivating a cell is offered using the above-mentioned oriented film or the above-mentioned base material for cell cultures of this invention.

[0016] According to still more nearly another side face of this invention, the cast of the hydrophobic organic solvent solution of the independent polymer which has (1) biodegradability and amphiphilic, or two or more polymer mixture is carried out on a substrate. It is made to dew on this cast liquid front face at the same time it transpires this organic solvent, and the manufacture approach of the oriented film containing process: which extends process; which extends a honeycomb structure object, and the honeycomb structure object of (2) above of this invention is offered by evaporating the minute waterdrop produced by this dew condensation.

[0017]

[Embodiment of the Invention] Hereafter, the embodiment and practice of this invention are explained to a detail. The oriented film of this invention carries out the cast of the hydrophobic organic solvent solution of the polymer mixture which consists of the independent polymer or biodegradability polymer which has biodegradability and amphiphilic, and an amphiphilic polymer on a substrate, it is made to dew on this cast liquid front face at the same time it transpires this organic solvent, and it is characterized by being obtained by extending the honeycomb structure object acquired by evaporating the minute waterdrop produced by this dew condensation.

[0018] In this invention, the mixture of two or more polymers which consist of the polymer which may use the independent polymer which has biodegradability and amphiphilic, or has biodegradability, and the polymer which has amphiphilic may be used.

[0019] As a biodegradability polymer which can be used by this invention, aliphatic series polycarbonates, such as polybutylene carbonate and polyethylene carbonate, etc. are desirable from a soluble viewpoint to an organic solvent in biodegradability aliphatic series polyester, such as polylactic acid, polyhydroxy butanoic acid, the poly caprolactone, a polyethylene horse mackerel peat, and a polybutylene horse mackerel peat, and a list. Especially, polylactic acid and the poly caprolactone are desirable from viewpoints, such as an ease of acquisition, and a price.

[0020] As an amphiphilic polymer which can be used by this invention It is desirable that there is no toxicity if it takes into consideration to use as a cell culture base material. A polyethylene glycol / polypropylene-glycol block copolymer, The amphiphilic polymer which makes an acrylamide polymer a principal chain frame and has a lactose radical or a carboxyl group as the dodecyl and a hydrophilic side chain as a hydrophobic side chain, Or the ion complex of anionic macromolecules, such as heparin, and dextran sulfate, a nucleic acid (DNA and RNA), and long-chain alkyl ammonium salt, It is desirable to use the amphiphilic polymer which made the hydrophilic radical water-soluble protein, such as gelatin, a collagen, and albumin.

[0021] Moreover, as an independent polymer which has biodegradability and amphiphilic, a polylactic acid-polyethylene-glycol block copolymer, a Pori epsilon-caprolactone-polyethylene-glycol block copolymer, a Pori malic-acid-Pori malic-acid alkyl ester block copolymer, etc. are

mentioned, for example.

[0022] In creating the honeycomb structure object of this invention, it is required to be nonaqueous solubility (hydrophobicity) as an organic solvent used from it being required to make a minute waterdrop particle form on a polymer solution. As an example of a hydrophobic organic solvent, nonaqueous solubility ketones, such as ester, such as aromatic hydrocarbon, such as halogen system organic solvents, such as chloroform and a methylene chloride, benzene, toluene, and a xylene, ethyl acetate, and butyl acetate, and methyl isobutyl ketone, a carbon disulfide, etc. are mentioned. These organic solvents may be used as a mixed solvent which combined these solvents, even if it uses it independently. The polymer concentration of the sum total of both biodegradability polymer and amphiphilic polymer which dissolves in a hydrophobic organic solvent is 0.01 to 10 % of the weight preferably, and is 0.05 to 5 % of the weight more preferably. The dynamics reinforcement of the film which will be obtained if polymer concentration is lower than 0.01 % of the weight runs short and is not desirable. Moreover, at 10 % of the weight or more, polymer concentration becomes [ polymer concentration ] high too much, and sufficient honeycomb structure is not acquired.

[0023] Moreover, although especially the presentation ratio is not limited when using a biodegradability polymer and an amphiphilic polymer, it is within the limits of 99:1-50:50 (wt/wt) preferably. When an amphiphilic polymer ratio is one or less, the stability of the honeycomb structure object with which it may become difficult with which for uniform honeycomb structure to obtain, and an amphiphilic polymer ratio is obtained or more by 50, and dynamic stability may fall especially.

[0024] In this invention, the cast of this polymer organic solvent solution is carried out on a substrate, and a honeycomb structure object is prepared. Liquids, such as a macromolecule which was excellent in organic solvent-proof nature, such as inorganic materials, such as glass, a metal, and a silicon wafer, polypropylene, polyethylene, and a polyether ketone, as a substrate, water, a liquid paraffin, and a liquefied polyether, can be used. Especially, when water is used for a base material, this structure can be independently taken out from a substrate easily by employing efficiently the independence nature which is the description of this honeycomb structure object, and it is suitable.

[0025] The device in which honeycomb structure is formed by this invention is considered as follows. When a hydrophobic organic solvent evaporates, in order to take the latent heat, the temperature of a cast philharmonic front face falls, and the drop of minute water condenses and adheres to a polymer solution front face. The surface tension between water and a hydrophobic organic solvent decreases, for this reason, it faces that a water particle tends to condense and it is going to become one lump, and work of the hydrophilic part in a polymer solution is stable. A solvent follows on evaporating, and it stands in a line in the form in which the drop which carried out the hexagonal form carried out the closest packing, and finally, water flies and it remains as a form where the polymer was regularly located in a line in the shape of a honeycomb.

[0026] therefore, as an environment where this film is prepared (1) Carry out the cast of the hydrophobic organic solvent solution on a substrate, and it is made to dew on this cast liquid front face at the same time it transpires this organic solvent by spraying high humidity air. In a list, How to evaporate the minute waterdrop produced by this dew condensation; (2) hydrophobic organic solvent solution Approach; which evaporates the minute waterdrop which carried out the cast on the substrate under atmospheric air of 50 - 95% of relative humidity, was made to dew on this cast liquid front face while transpiring this organic solvent, and was produced by this dew condensation is desirable. Thus, although not limited, especially the magnitude of each of the made honeycomb structure objects (each) is 0.1 to 100 micrometers preferably, is 0.1 to 10 micrometers more preferably, and if it is the magnitude of this range, it can be suitably used as a base material for cell cultures.

[0027] The oriented film of this invention is obtained by extending the honeycomb structure object acquired as mentioned above. Especially the approach of a drawing can be performed by not being limited, for example, pulling two or more edges of a honeycomb structure object film in a tongue and the expanding direction by the pincette or the hand. Or it can also extend using a micro manipulator.

[0028] In this invention, any of uniaxial stretching, biaxial stretching, or a triaxial drawing are sufficient as a drawing. The mimetic diagram of the example of the drawing in this invention is shown in drawing 1. In drawing 1, uniaxial stretching and (b) show biaxial stretching, (c) shows a triaxial drawing, (a) shows the angle on which a symmetry axis and the drawing direction make  $\alpha$ , and  $\beta$  and  $\gamma$  show the angle which the drawing direction makes. In this invention, although especially the rate of expanding of the drawing direction is not limited, it is within the limits of 1.1 to 10 times preferably. At 1.1 or less times, the effectiveness of this invention according [ the rate of expanding ] to a drawing is small, and the rate of expanding becomes [ a film ] is easy to be destroyed by 10 or more times.

[0029] The oriented film of this invention produced as mentioned above can be used as a base material for cell cultures, and can cultivate a cell using this base material. Especially the class of cell which can be cultivated using the oriented film of this invention is not limited, but can cultivate the cultured cell of arbitration, the cell extracted from the organization in the list.

[0030] Especially the class of culture medium used for a cell culture is not limited, but can choose suitable culture media (for example, culture medium which added fetal calf serum etc. to a Williams'E culture medium, F-10 culture medium, RPM11640 culture medium, the MEM culture medium of Eagle, DMEM culture media, or these culture media) according to the class of cell. A culture condition can be suitably chosen according to the class of cell, and, generally can be cultivated under conditions, such as pH 6-8, the temperature of 30-40 degrees C, and the 5% CO<sub>2</sub> existence lower.

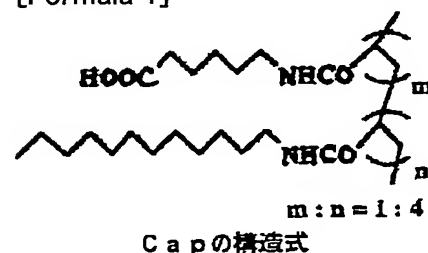
[0031] A three-dimension organism can also be formed from a cell, using the base material for cell cultures of this invention. Moreover, especially the base material for cell cultures of this invention can control the array of a cell, and is available to a rebirth of the organization which has the array structure of a cell like the cardiac muscle tissue or a blood vessel organization especially. Hereafter, although this invention is explained to a detail using an example, this invention is not limited at all by the example.

[0032]

[Example] Example 1: 1:2.5:6.5 mixed comparatively (volume ratio) the chloroform solution (10g/L) of a Pori epsilon-caprolactone (molecular weight: 100,000-190,000), the benzene solution (0.4 g/L) of the amphiphilic giant molecule Cap (the chemical structure is indicated below), and benzene, and the polymer solution was prepared. It changed into the condition that develop a flare on a glass petri dish with a diameter of 9cm, develop 90micro of polymer solutions L for Milli-Q water uniformly on this water surface, and a drop does not spread. After checking that the solvent had evaporated, the cast of the 150micro of the same solutions L was carried out on the water surface, and the honeycomb film (diameter: 2cm) was obtained by spraying high humidity air ( drawing 2 ). The ends of the honeycomb film which has floated on the water surface were extended to a tongue and 1 shaft orientations with the pincettes, and the oriented film (major-axis:3.4cm and minor-axis:1.8cm) was obtained. This was moved on the glass plate. The array structure of the pore elongated in the optical microscope photograph ( drawing 2 ) of an oriented film was accepted.

[0033]

[Formula 1]



[0034] The example 1 of a trial: The oriented film obtained in the example 1 was installed on the glass substrate, and the rat embryo heart origin cardiac muscle cell was cultivated on this. Culture was performed using F10 culture medium within the CO<sub>2</sub> incubator (CO<sub>2</sub> concentration

=5%, temperature =37 degree C, relative humidity = 80%). Seeding of the cardiac muscle cell was carried out to what installed the non-extended honeycomb structure object film on the glass substrate as an example of a comparison, and the thing which installed the PCL-amphiphilic macromolecule cast film without pore on the glass substrate, and it cultivated on the same conditions.

[0035] The result of having taken an optical microscope photograph is shown in drawing 2 R> 2 after culture. When the oriented film obtained in the example 1 was used so that the result of drawing 2 may show, the cell was extended and arranged to the specific one direction, and the direction was in agreement in the drawing direction of a film. Moreover, in the case of the example of a comparison, regularity was not accepted in the expansion and the array direction of a cardiac muscle cell.

[0036]

[Effect of the Invention] It became possible to offer the cell culture base material using the oriented film and it which can control the array of an oriented film available for formation of a three-dimension organism and the cell culture base material using it, especially a cell from culture of a cell, and a cell by this invention. The oriented film of this invention and the cell culture base material using it are available to a rebirth of the organization which has the array structure of a cell like the cardiac muscle tissue or a blood vessel organization especially, and can be applied to an extensive cell, and/or culture and playback of an organization.

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DESCRIPTION OF DRAWINGS

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[Brief Description of the Drawings]

[Drawing 1] Drawing 1 is drawing showing the format of extension of a honeycomb structure object.

[Drawing 2] Drawing 2 shows the fluorescence microscope photograph (upper right: oriented film of a Pori epsilon-caprolactone honeycomb structure object and a lower right:Pori epsilon-caprolactone honeycomb structure object) of the cardiac muscle cell cultivated on these structures in the optical microscope photograph of a Pori epsilon-caprolactone honeycomb structure object (upper left) and its extension structure (lower left), and the list.

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## DRAWINGS

[Drawing 1]

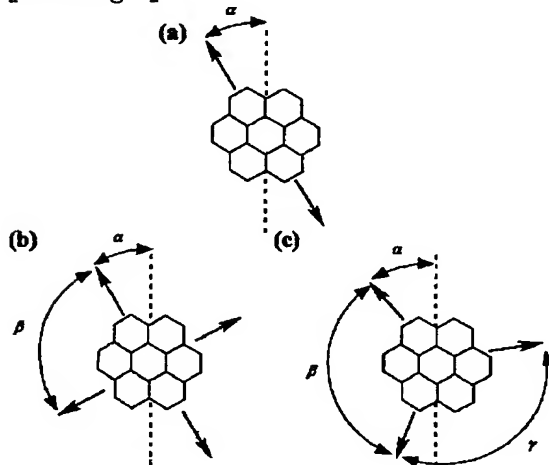


図1 ヘキサゴン構造体の延伸の様式。(a)一軸延伸、(b)二軸延伸、(c)三軸延伸。  
 $\alpha$ は対称軸と延伸方向のなす角。 $\beta$ 、 $\gamma$ は延伸方向のなす角。

[Drawing 2]

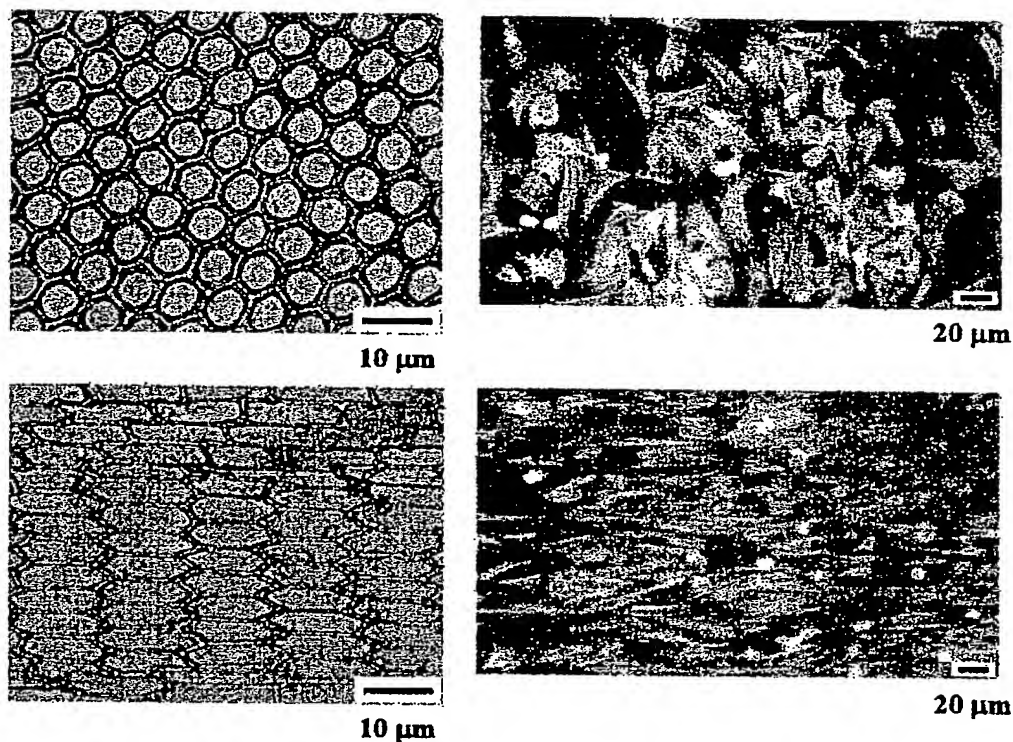


図2 ポリ-ε-カプロラクトンハニカム構造体（左上）および、その延伸構造体（左下）の光学顕微鏡写真。これら構造体の上で培養した心筋細胞の蛍光顕微鏡写真（右上：ポリ-ε-カプロラクトン構造体ハニカムフィルム上、右下：延伸構造体フィルム上）。

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